Therapeutic Targeting of Vascular Senescence Pathways

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ABSTRACT

Blood vessels become less flexible with age. Arteries narrow and become less flexible, hindering blood circulation in those with various vascular diseases including atherosclerosis. Mechanistically, vascular senescence plays important roles in the pathogenesis of normal aging and age-related cardiovascular diseases. Vascular senescence also causes vascular dysfunction, resulting in damage to the vessel wall. This review presents the various molecular biologic mechanisms of vascular senescence and their therapeutic implications. We discuss topics such as mitochondrial oxidative stress/anti-oxidant enzymes, nitric oxide synthase–nitric oxide signaling and the Akt-mTORC1-S6K1 pathway and sirtuin family members.

keywords: Vascular diseases; Vascular senescence; Mitochondrial oxidative stress/anti-oxidant enzymes; NOS-NO; Akt-mTORC1-S6K1; Sirtuin

INTRODUCTION

Healthy blood vessels possess elasticity, which facilitates efficient blood flow through intricate turns and branches. However, blood vessels stiffen with age, thereby reducing blood flow and pushing the heart to work harder in older individuals and in patients with vascular diseases such as atherosclerosis. Vascular senescence is linked to age-related vascular diseases. Aging vascular endothelium is susceptible to the development of various vascular diseases including cardiovascular disease, peripheral vascular disease, diabetic retinopathy, renal vascular disease and micro-vascular disease. High-fat diets promote atherogenesis via mechanisms involving premature senescence in vascular cells, which leads to vascular dysfunction, an imbalance between vasodilation and vasoconstriction.

Similar to other cell types, vascular cell senescence can be detected by senescence-associated beta-galactosidase staining or by examination of typical protein markers of senescence such as p53 and p21. The enhanced expression of plasminogen activator inhibitor-1, Intercellular Adhesion Molecule-1 **(ICAM-1)** and Vascular Cell Adhesion Molecule-1 **(VCAM-1)** can serve as biomarkers of vascular cell-specific senescence [1-4]. Recently, the oncogenic serine/arginine-rich splicing factor-1 was shown to translocate from the nucleus to the cytosol under endothelial senescence conditions and to modulate the expression of critical regulators of vascular physiology including Vascular Endothelial Growth Factor **(VEGF),** endoglin, Tissue Factor **(TF)** and lamin A, which is another useful marker for vascular senescence [5]. Moreover, decreased proliferative potential and increased Reactive Oxygen Species **(ROS)** levels can be detected in senescent vascular cells. These changes trigger pathophysiological consequences including decreased vascular integrity and vasodilation as well as increased vascular inflammation, atherogenesis and resultant thrombosis.

Several factors induce premature vascular senescence in vascular endothelial cells or in vascular smooth muscle cells. For example, oxidative stress-induced DNA damage increases with aging and plays an important role in the induction of vascular senescence followed by atherosclerosis [6]. In addition, arterial components of angiotensin II signals increase with aging and contribute to the senescence-associated pathogenesis of atherosclerosis [7,8]. In contrast, endothelial production of vasodilators such as Nitric Oxide **(NO)** and prostacyclin exert a protective role on the vessel wall, thereby inhibiting vascular senescence [9-13].

Here, we describe various components involved in vascular senescence. In addition, we describe possible treatment strategies for vascular senescence.

MECHANISMS UNDERLYING VASCULAR SENESCENCE

Vascular senescence involves the senescence of endothelial and vascular smooth muscle cells. Two types of vascular senescence-replicative senescence with telomere attrition and stress-induced premature senescence without telomere involvement-induce vascular cell growth

arrest and loss of vascular homeostasis, which contribute to the initiation and progression of cardiovascular diseases. Several signaling mechanisms are involved, which can lead to deleterious or beneficial effects in aging-related vascular diseases.

Oxidative Stress

Oxidative stress can induce various types of DNA damage in cells. Vascular endothelial cells treated with chronic oxidative stress inducers, such as oxidized Low-Density Lipoprotein (LDL), display shorter telomeres, promoting senescence [14,15]. Vascular smooth muscle cells undergo telomere-based senescence in atherosclerosis [16]. Moreover, oxidative stress can induce vascular cell senescence in a telomere-independent manner [8].

Besides inflicting direct damage to DNA, oxidative stress can induce atherosclerosis in additional ways. For example, ROS inhibit telomerase and promote senescence by suppressing the PI3K/Akt pathway [17]. In addition, ROS induce the translocation of telomerase from the nucleus to the cytosol, preventing the interaction of telomerase with the telomere [18]. ROS-induced p53 activation reduces survival signals induced by Insulin-Like Growth Factor-1 (IGF-1) receptor in vascular smooth muscle cells [19]. In addition, ROS facilitate LDL uptake by vascular cells by increasing the number of receptors [20,21]. Absorbed oxidized LDL causes oxidative stress by enhancing ROS or reducing NO generation.

Interestingly, continuous mitogenic stimuli induced by Ras, Rac1 or Akt cause stress-induced premature senescence in vascular cells [22]. These phenomena are attributed to the dysregulation of oxidation-reduction reactions followed by ROS-induced p53 activation. From a pathological point of view, vascular Akt or Ras signals are activated in pro-atherogenic conditions such as chronic inflammation, hyperinsulinemia and hypercholesterolemia [22]. Age-related increases in arterial expression of angiotensin II, a potent mitogen that can activate Ras, can also contribute to atherosclerosis [23]. Conversely, the suppression of angiotensin II activity lowers both the prevalence and mortality rates in individuals with cardiovascular diseases [24]. Furthermore, angiotensin II can induce premature senescence of vascular smooth muscle cells and accelerate the development of atherosclerosis through p53/p21 activation [7].

NO And NO Synthase (NOS)

Functional Endothelial NOS (eNOS) oxidizes its substrate L-arginine to form L-citrulline and NO. Vascular endothelial cells express eNOS, thereby producing NO, which plays an important physiological role in cardiovascular remodeling and function. Under normal conditions, eNOS activity is suppressed. However, when eNOS is activated by acetylcholine, bradykinin, thrombin or histamine, it produces NO, which promotes vasodilation. Vasoconstriction can occur with aging and may be due to decreased eNOS expression and NO production. While the detailed mechanisms underlying decreased eNOS expression and NO production remain unclear, various proteins (such as caveolin-1, Akt and Hsp90), estrogen and several growth factors are believed to be involved.

Levels of eNOS are regulated by Dimethylarginine **(DDAH)-**Asymmetric Dimethylarginine **(ADMA)** signals. ADMA, an analog of L-arginine, acts as an endogenous eNOS inhibitor that reduces NO production. Generally, ADMA levels tend to be upregulated in patients with cardiovascular diseases such as hypertension, atherosclerosis and hyperlipidemia [25,26]. ADMA-mediated downregulation of NO can promote blood cell adhesion to vessel walls. ADMA is negatively regulated by its major hydrolase, DDAH. DDAH maintains normal remodeling and vascular cell activity by eliminating ADMA. Therefore, high ADMA concentrations and downregulation of DDAH activity might be important risk factors leading to cardiovascular disease pathologies. Several studies have reported that DDAH–ADMA signaling plays important roles in maintaining vascular homeostasis through the regulation of vascular motility and neovascularization [27,28].

Notably, not all eNOS activated by DDAH–ADMA exerts beneficial effects on vessels. For example, although O_2^- is subjected to detoxification by serial action of Manganese Superoxide Dismutase **(MnSOD)** and catalase, sometimes it reacts with NO under NO-rich conditions, forming the potent free radical peroxinitrite **(ONOO⁻)** [29]. ONOO⁻ is injurious to macromolecules and suppresses the activities of MnSOD and eNOS in vascular endothelial cells [30]. Under these circumstances, eNOS shifts from being a beneficial enzyme to a harmful enzyme that produces ROS, which induce oxidative stress in senescent vascular cells. However, it remains unclear whether the functional change in eNOS is the cause or result of vascular senescence.

The conventional theory that NO inhibits vascular senescence via activation of telomerase followed by maintenance of telomere length has been challenged by the results of multiple experiments. For example, neither eNOS activity nor NO levels affect telomerase activity [31]. Instead, Sirt1 might be the efficient target protein involved in NO-mediated anti-senescence effects in vascular cells. In fact, NO donors can increase Sirt1 expression and inhibit oxidative stress-induced senescence [32]. Moreover, NO donor-mediated anti-senescence effects are efficiently inhibited by the downregulation of Sirt1 expression [32]. Therefore, NO seems to exert anti-senescence action through Sirt1 activation and scavenging intracellular stresses.

Akt-Mtorc1-S6K1 Pathways

Akt, mTORC1 and downstream S6K1 act as sensors of energy, nutrients and stress. As such, they are important proteins involved in cellular growth and metabolism. Oncogenic Akt is important for the regulation of vascular function. Transient overexpression of Akt mediates VEGF-induced vascular cell proliferation and migration and phosphorylates and activates eNOS, thereby inducing vasodilation and protecting the vasculature [33]. However, chronic expression of Akt exerts the opposite action: induction of vascular senescence and dysfunction. For example, Wang et al. reported that chronic activation of Akt and mTORC1 is implicated in senescence-associated diabetic vascular diseases [34].

Although mTORC1-S6K1 is implicated in cardiac hypertrophy [35], little is known about the possible involvement of mTORC1-S6K in cardiac senescence. However, the role of mTORC1-S6K1

in vascular senescence has been well studied. First, the activity of mTORC1-S6K1 is upregulated in senescent vessels [35], indicating that this signal might play an important role in vascular senescence. The most important effect of this signal in vascular senescence is to suppress NO production and enhance ROS production, thereby inducing vascular senescence [35]. Moreover, mTORC1-S6K1 is believed to induce eNOS uncoupling, in which eNOS produce ROS, rather than NO, through an undefined mechanism, thereby increasing ROS and inducing vascular senescence [35]. Additionally, mTORC1-S6K1 induces adhesion molecules such as ICAM-1, VCAM-1 and TF protein [35]. Collectively, these effects of mTORC1-S6K1 have the potential to cause senescence-associated cardiovascular diseases.

Interestingly, mTORC1 and S6K1 do not necessarily cooperate with each other to deliver the same signal [35,36]. In fact, these proteins exert opposite effects in regulating TF [35,36]. Whereas mTORC1 suppresses vascular TF expression, S6K1 is involved in enhancing TF expression.

Sirtuin Family Members

Sirtuins are evolutionarily conserved enzymes that possess either NAD^+-dependent deacetylase or mono-ADP-ribosyltransferase activity. Sirtuins influence a wide range of cellular processes including aging, transcription, apoptosis, inflammation, stress resistance, energy metabolism, circadian clocks and mitochondrial biogenesis. Sirtuins are important proteins implicated in age-related diseases such as cancer, Alzheimer's disease and cardiovascular diseases as well as in lifespan extension and mitochondrial metabolism. In addition, these proteins have various beneficial activities in the vascular system such as cell protection and promotion of remodeling through the inhibition of oxidative stress and inflammation. The effects of sirtuins on cardiovascular diseases have been elucidated mainly through studies on genetic knockout in mice. Among the seven mammalian sirtuin family members, Sirt1, a NAD^+-dependent deacetylase, exerts protection from cardiovascular diseases by deacetylating various target proteins.

Sirt1 exerts potent protective activity against vascular senescence. Overexpression of Sirt1 in vascular cells can inhibit oxidative stress-induced premature senescence by deacetylating and inhibiting p53 [37]. Mice overexpressing Sirt1 show increased glucose tolerance due to metabolic activation accompanied by decreased blood cholesterol, adipokine, insulin and fasting glucose levels [38]. Uptake of the Sirt1 activator resveratrol can suppress vascular senescence-inducing signals in high-calorie-fed mice [39]. Suppression of Sirt1 activity leads to impairment in angiogenesis or ischemia-induced neovascularization [40]. Sirt1 decreases mitochondrial ROS generation and increases vascular NO through deacetylation of eNOS [41,42]. Collectively, these observations suggest that Sirt1 can increase vascular cellular lifespan and organismic lifespan through diverse mechanisms.

The effects of Sirt1 on cardiac senescence are less well known than are the effects of Sirt1 on vascular senescence. However, given that the expression of Sirt1 induced in the heart attenuates age-related myocardial hypertrophy, apoptosis of cardiomyocytes and cardiac dysfunction

[43], Sirt1 might have protective effects in the heart. Sirt1-induced deacetylation of FoxO family members seems to be involved in the protective role of Sirt1 in the heart. However, further studies are required to unveil the potential role of Sirt1 in the cardiac system.

The effects of other sirtuin family members on cardiovascular diseases are currently being explored, especially in studies involving Sirt3 and Sirt7. For example, Sirt3 can protect vascular cells from oxidative stress and lower the risk of age-related cardiovascular diseases [44,45]. Given that increased oxidative stress and downregulated Sirt3 activities were easily detected in senescent vascular cells, it can be postulated that Sirt3 acts as a marker of the extent of senescence and that regulation of Sirt3 is a potential target for aging-related cardiovascular disease therapies. Sirt7 prevents the age-related functional decline in the heart. Knockout of this gene caused cardiac hypertrophy, fibrosis, lipofuscin accumulation, inflammatory cardiomyopathy and apoptosis of cardiomyocytes, conditions that worsen with age [46]. Like Sirt1, Sirt7 can inhibit oxidative stress- and aging-related cardiac dysfunction by deacetylating and inhibiting p53 and FoxO1 proteins [47]. Compared with wild-type mice, both Sirt3 knockout mice and Sirt7 knockout mice showed cardiac hypertrophy phenotypes [46,48], indicating that these proteins contribute to the maintenance of heart function.

Although the effects of sirtuins have been investigated in cardiovascular diseases, the molecular mechanisms underlying sirtuin-mediated regulation of heart and vascular systems remain unclear. Sirtuins are involved in the beneficial effects of caloric restriction or resveratrol on cardiovascular diseases, suggesting that the roles of these enzymes in vascular system health warrant further study.

TREATMENT STRATEGIES AGAINST VASCULAR SENESCENCE-RELATED DISEASES

Investigating the inhibiting or activating pathways underlying vascular senescence in the biological setting may be an effective strategy in the treatment of age-related cardiovascular diseases. Several promising therapeutic strategies against age-related vascular diseases are discussed below.

Anti-Oxidant Enzymes

Naturally occurring anti-oxidants such as ferritin or glutathione are significantly decreased in vascular senescence or atherosclerosis conditions. However, the ability of anti-oxidants to suppress atherogenesis has been somewhat controversial in various studies. It is generally believed that anti-oxidants show no protective role in higher animals, and that dietary intake of anti-oxidants is ineffective in reducing cardiovascular diseases [49,50]. However, the enhancement of anti-oxidant enzyme activities is currently an intriguing research topic. For example, expression of Nrf2, an important transcription factor involved in anti-oxidant responses, reduces oxidative stress or inflammation in vascular cells [51,52]. Super Oxide Anion **(O_2^-)** is the most harmful ROS

in vascular cells as well as in other cell types. The mitochondrial anti-oxidant enzyme MnSOD weakens the toxicity of O_2^- by catalyzing the rapid conversion (dismutation) of O_2^- into Hydrogen Peroxide **(H_2O_2)** and Oxygen **(O_2)**. Another mitochondrial anti-oxidant enzyme, catalase, eliminates the toxicity of H_2O_2 by inducing the reduction of H_2O_2 into water and O_2. The sequential attenuated toxicity and detoxification-mediated activities of MnSOD and catalase, respectively, are important defense mechanisms in protecting vascular cells from oxidative stresses.

NOS-NO Utilization

As mentioned above, eNOS catalyzes the production of NO and L-citrulline from L-arginine. Multiple levels of the arginine-NOS-NO pathway can be utilized for targeted therapies against vascular senescence [53]. One option is to increase the NOS substrate availability via L-arginine supplementation or arginase inhibitors. A second option is to increase NOS expression via gene transfer. A third strategy would be to deliver NO gas directly via inhalation or pharmacological donors. In the cardiovascular system, Cyclic Guanosine Monophosphate **(cGMP)** signaling is essential for endothelial, vascular smooth muscle, and cardiac myocyte function. Particularly, NO-induced vascular vasodilation (relaxation) is associated with increased levels of cGMP in vascular smooth muscle cells [54]. NO regulates vasodilation/relaxation via effects on Soluble Guanylate Cyclase **(sGC)** to produce cGMP from guanosine triphosphate [55]. Like Cyclic Adenosine Monophosphate **(cAMP)**, cGMP causes smooth muscle relaxation. cGMP can be hydrolyzed into GMP by cyclic nucleotide Phosphodiesterases **(PDEs)** [55]. Therefore, an additional strategy is to use GC activators such as a riociguat or to employ phosphodiesterase inhibitors such as sildenafil.

Several flavonoids are activators of NOS, triggering a NO boost. For example, native cocoa beans help activate NOS and produce NO, which helps maintain low blood pressure [56]. Garlic, a natural activator of NOS [57], is believed to be beneficial to the cardiovascular system by decreasing blood pressure, inhibiting platelet aggregation and reducing blood fat and cholesterol [58]. Allicin and ajoene, which are organosulfur compounds in garlic, are believed to exert such beneficial properties.

Inhibitors of Mtorc1-S6K1

Given the effects of mTORC1-S6K1 on vascular senescence, inhibition of mTORC1 using rapamycin and inhibition of S6K1 using resveratrol might have beneficial effects in treating aging-associated cardiovascular diseases.

Resveratrol, a natural polyphenol, appears to inhibit senescence and protect from age-related vascular diseases and diabetes. Resveratrol has anti-aging effects in vascular cells by suppressing the expression of ICAM-1 and VCAM-1 induced by high glucose and TNF-α or by inducing eNOS recoupling followed by suppression of ROS generation.

Rapamycin is thought to increase lifespan or inhibit age-related diseases such as cardiovascular diseases. However, inhibition of mTORC1-S6K1 using rapamycin or its analog everolimus in

cultured young vascular cells promotes vascular senescence [59]. Therefore, multi-directional studies including genetic studies, pharmacological studies and comparative investigations into the function of mTORC1-S6K1 between young and aged vascular cells are required. Moreover, as mentioned earlier, mTORC1 and S6K1 sometimes exert opposing effects; therefore, the effects of each protein on age-related cardiovascular diseases or vascular senescence must be investigated and more selective drugs that preferentially inhibit S6K1 rather than mTORC1 are required.

Sirtuin Activators

Sirtuin activators have been shown to mimic caloric restriction and delay aging in metazoans [60]. Therefore, sirtuin activators are of particular interest among researchers as molecules involved in increasing lifespan and cell survival and in preventing aging-related diseases such as vascular disease. In parallel, Sirt1 is the most highly investigated member of the sirtuin family, as it plays an important role in several processes including mitochondrial metabolism and stress response. Therefore, a variety of approaches using natural or synthetic activators of sirtuin have focused on targeting Sirt1. Resveratrol, a type of natural polyphenol found in red wine, mimics caloric restriction to activate longevity genes. Resveratrol, the most powerful Sirt1 activator known, might be an effective way to activate Sirt1 *in vivo* and promote beneficial health effects, most of which resemble the effects of Sirt1 over expression. Treatment of endothelial cells with resveratrol increased the angiogenic response of endothelial cells [40], indicating that some members of the sirtuin family are important modulators of endothelial angiogenic functions. Activation of Sirt1 by resveratrol also induces Krüppel-like factor 2 expression, conferring an endothelial vasoprotective phenotype [61]. Resveratrol induces vascular smooth muscle cell differentiation through stimulation of Sirt1 and AMPK [62]. Moreover, resveratrol induces vascular growth factor and strengthens capillary network formation in cardiac muscle. High fructose-induced vascular dysfunction could also be improved by resveratrol treatment [63]. However, due to its poor bioavailability, several reformulated versions of resveratrol, such as SRT501 and resVida, have been developed with improved bioavailability.

Molecules that are structurally unrelated to resveratrol have been developed that activate sirtuin activities more potently than does resveratrol. For example, synthetic Sirt1 activator SRT1720 is an experimental drug that was intended as a small-molecule activator of the sirtuin subtype Sirt1. Like Sirt1, SRT1720 seems to have beneficial effects on health in that it extends lifespan and improves the health of mice fed a standard diet [64]. SRT1720 ameliorates vascular endothelial dysfunction with aging in mice by enhancing Cox-2 signaling and reducing oxidative stress and inflammation [65]. It also substantially attenuates angiotensin II-induced atherosclerosis in apoE-/- mice by inhibiting the vascular inflammatory response [66].

According to Pacholec et al., both SRT1720 and resveratrol are not direct activators of Sirt1 [67]. SRT1720 and resveratrol do not lead to the activation of Sirt1 with native peptide. Furthermore, SRT1720 neither lowers plasma glucose nor improves mitochondrial capacity in mice fed a

high-fat diet. Since resveratrol, SRT1720 and its structurally related compounds (SRT2183 and SRT1460) exhibit multiple off-target activities, additional specific and direct activators need to be developed for the efficient activation of Sirt1.

PERSPECTIVES AND CONCLUSIONS

An important hallmark of vascular aging is cellular senescence of vascular endothelial and vascular smooth muscle cells. Age-related physical or functional changes in blood vessels lead to decreased vascular compliance and increased vascular inflammation, which collectively cause atherogenesis. To induce positive health benefits in patients with vascular aging, various therapeutic approaches are being developed to target the signaling molecules affecting vascular senescence (Figure 1).
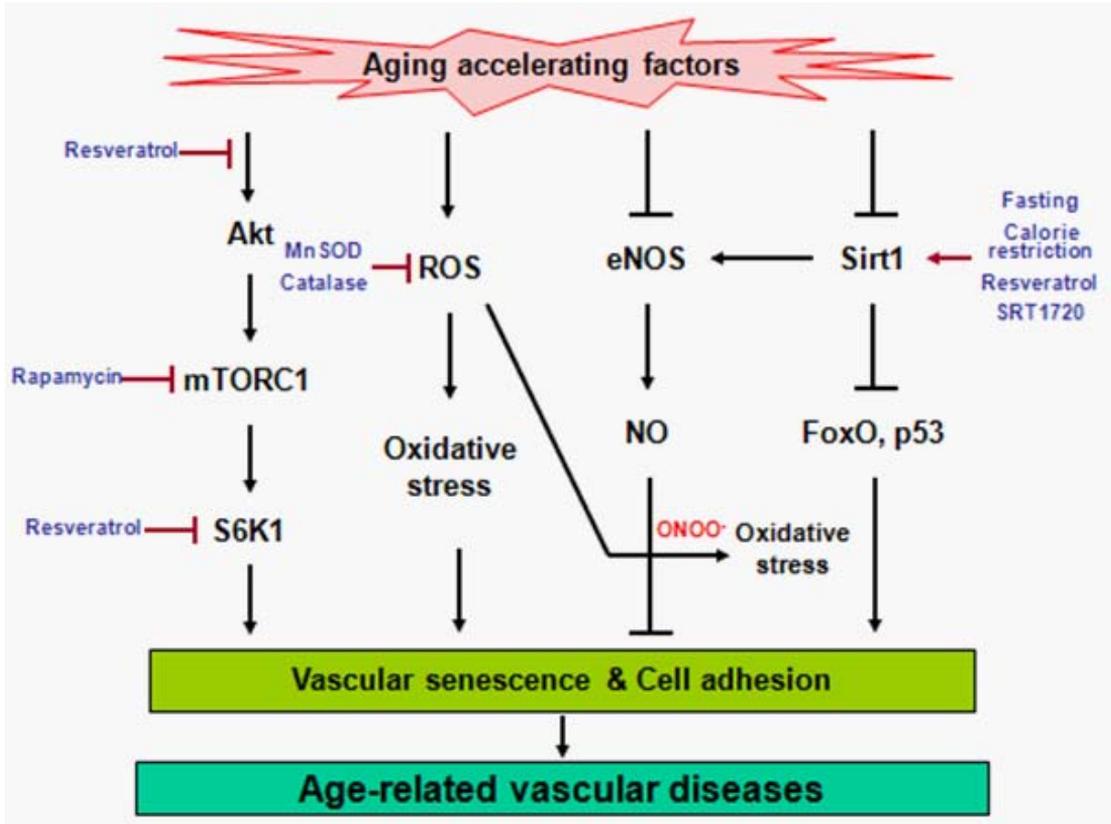

Figure 1: Targeted therapies utilizing various mechanisms involved in vascular senescence in age-related diseases.

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